# Managing sulfur metabolism in plants

Hawkesford, M.J.; De Kok, LJ

*Published in:*
Plant cell and environment

*DOI:*
10.1111/j.1365-3040.2005.01470.x



*Publication date:*
2006

*Plant, Cell and Environment* (2006) **29**, 382–395 doi: 10.1111/j.1365-3040.2005.01470.x

# Managing sulphur metabolism in plants

MALCOLM J. HAWKESFORD[1] & LUIT J. DE KOK[2]

[1]*Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK and*
[2]*Laboratory of Plant Physiology, University of Groningen, 9750 AA Haren, the Netherlands*

## ABSTRACT

**Resolution and analysis of genes encoding components of the pathways of primary sulphur assimilation have provided the potential to elucidate how sulphur is managed by plants. Individual roles for members of gene families and regulatory mechanisms operating at gene, cellular and whole plant levels have been recognized. Sulphur is taken up and transported around the plant principally as sulphate, catalysed for the most part by a single gene family of highly regulated transporters. Additional regulation occurs in the pathway of reduction of sulphate to sulphide and its incorporation into cysteine, which occurs principally within the plastid. Cellular and whole-plant regulation of uptake, and the assimilatory pathway attempt to balance supply with demand for growth and include mechanisms for remobilization and redistribution of sulphur. Furthermore, optimization of sulphur assimilation requires coordination with carbon and nitrogen pathways, and multiple processes have been proposed to contribute to this balance. Present studies on *cis* and *trans* elements are focusing on transcriptional regulation, but this regulation still needs to be linked to apparent metabolite sensing. Whilst the components of the assimilatory pathways have been resolved after many years of controversy, uncertainties remain concerning roles of individual genes in gene families, their sub-cellular localization and their significance in balancing sulphur flux to sulphur demand of the plant for growth under variable environmental conditions.**

*Key-words*: cysteine synthesis; metabolic regulation; nutrition; sulphate transporters.

## INTRODUCTION

The focus of this review is to summarize the new developments and key open questions in the current state of understanding of regulation of sulphur metabolism in plants. An integration of ideas from the level of the control of gene expression to the regulation of whole plant demand and partitioning of sulphur is presented. How a plant effectively manages one nutrient, in this case sulphur, may be a paradigm for how plants manage nutritional resources in general.

*Correspondence: Malcolm J. Hawkesford.*
*E-mail: malcolm.hawkesford@bbsrc.ac.uk*

There has been substantial progress on the elucidation of the pathways involved in sulphur uptake and assimilation with several comprehensive reviews appearing in recent years (Leustek 1996; Bick & Leustek 1998; Leustek & Saito 1999; Hawkesford & Wray 2000; Leustek *et al*. 2000; Saito 2000; Hell & Hillebrand 2001; Droux 2004; Saito 2004; Hawkesford 2005). Furthermore, as a result of recent transcriptome studies, an additional apparent complexity in plant responses to sulphur nutrition has been documented (Hirai *et al*. 2003; Maruyama-Nakashita *et al*. 2003; Nikiforova *et al*. 2003). There are complex responses for the adaptation to available sulphur supply to fulfil the demands for growth, and the interactions between shoot and root in the regulation of sulphur assimilation versus uptake and distribution are still poorly understood. Responses to sulphur deprivation (starvation) have been extensively studied; however, the limitation with this approach is that it represents a gross change of circumstance from luxurious abundance to severe limitation. Conversely, in some circumstances, sulphur may be present in abundance, and regulatory mechanisms will favour a limitation of uptake and assimilation. In most natural and agronomic scenarios, a steady-state acclimatization to an intermediary abundance is likely, and demands will depend on a variety of additional factors, both environmental and developmental. Responses to insufficient sulphur availability to match demand may be graded into initial responses specific for sulphur nutrition, followed by more complex responses which may be less specific and invoked by other nutrient deficiencies depending on circumstance (Fig. 1). The complexity of the adaptation is confirmed by the large numbers of nutrient-responsive genes observed in the transcriptome studies and in the numbers of interacting pathways that can be discerned when combined with additional data sets (Nikiforova *et al*. 2003, 2005; Hirai *et al*. 2004). The large numbers of regulated processes, although heavily interconnected, form a logical and sequential response and will involve both overlapping and discrete regulatory signals and pathways. Complex responses, such as a shift in the shoot:root ratio (Clarkson, Saker & Purves 1989), or changes in root architecture are likely to involve changes in expression of many genes. Proliferation of roots occurs in response to localized nutrient availability or there may be general root proliferation in response to limiting nutrient availability. *Arabidopsis* plants grown on low sulphur have an increased lateral proliferation (Kutz *et al*. 2002), possibly linked to a sulphur limitation-induced nitrilase

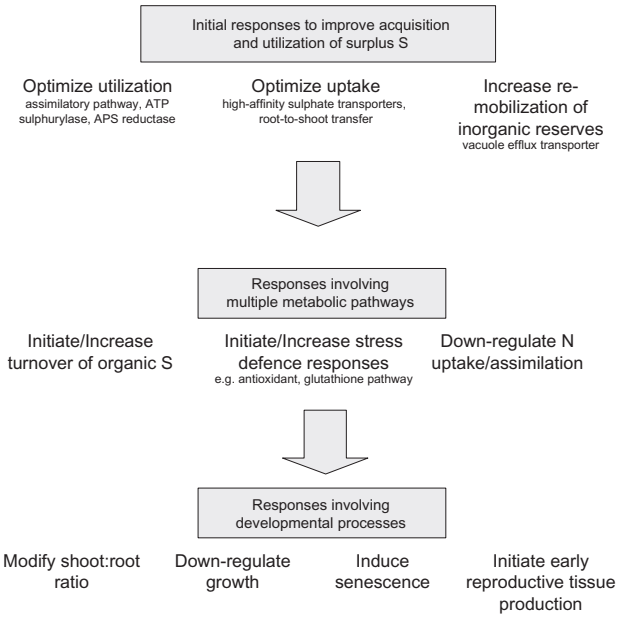


**Figure 1.** A hypothetical sequence of responses occurring upon limitation of sulphur supply to a flowering plant. APS, adenosine phosphosulphate.

which in turn may contribute to the control of indole-3-acetic acid (IAA) levels.

Although root plastids contain all of the enzymes of the sulphate assimilatory pathway, it is evident that the sulphate assimilated in the shoot chloroplasts is the primary source of reduced sulphur in the plant. Multiple points of control within the sulphate assimilatory pathway have been described with much emphasis on the cellular control of flux through the pathway (Vauclare *et al*. 2002; Wirtz, Droux & Hell 2004). Recent research has begun to determine the molecular components of the signal transduction pathway responsible for the transcriptional regulation controlling the expression of sulphate transporters (Maruyama-Nakashita *et al*. 2005). This review attempts to put these levels of control in the context of whole plant sulphur nutrition.

## THE NEED TO ADAPT TO AND MANAGE SULPHUR SUPPLY

In many industrialized regions, anthropogenic sulphur emissions have been restricted in recent years as a result of environmental legislation. A direct consequence has been a decrease in aerial dry and wet deposition from levels that were previously adequate to supply the sulphur needs of most crops to suboptimal inputs. Added to this is the use of high-analysis triple superphosphate fertilizers with no sulphur content, and the net result has been an increasing incidence of sulphur deficiency in crops worldwide. Sulphur deficiency decreases yields and has consequent effects on nitrogen uptake efficiency as well as specific effects on quality in terms of sulphur amino acid (and hence, protein) content. Decreased content of certain sulphur-rich proteins in wheat has detrimental impacts on flour and baking quality (Zhao, Hawkesford & McGrath 1999). In crops, remedial action is best achieved by early diagnosis, and novel protocols for early detection to supplant difficult visual diagnosis have evolved from research on metabolite responses to sulphur limitation (Blake-Kalff *et al*. 2000).

All plants regulate sulphur uptake and are able to adapt to a variable or fluctuating sulphur supply, for example:

1. *Acquisition when sulphur is in short supply*. This is probably a common scenario in many environments, and increasingly a situation occurring in agro-ecosystems if no sulphur fertilizer is provided.
2. *Redistribution around the plant*. Re-mobilization of reserves and redistribution around the plant are employed to maximize the usefulness of a limited resource. In cereals during grain filling, sulphur along with nitrogen is re-mobilized from vegetative tissues to the grain as vegetative tissues senesce. Redistribution of protein sulphur is driven by the need to redistribute nitrogen; however, in some circumstances, redistribution can occur as a direct result of sulphur limitation. Redistribution is important if the overall supply is limiting or if the supply is intermittent.
3. *Avoiding excess uptake*. This is a well-studied scenario in the laboratory which at cellular level involves the repression of uptake and assimilation. The rationale is to avoid excess uptake, which is energetically wasteful and/or to avoid potential osmotic imbalances. This is not always successful as with a high external sulphate supply, homeostasis may be overwhelmed. In some plants, for example in *Brassica*, there is a high demand for sulphur, and the sulphate content of vegetative tissues tends to be high.

## BALANCING SULPHUR NEEDS FOR THE WHOLE PLANT

The sulphur demand varies strongly between species and may be dependent upon the developmental stage of the plant (vegetative growth, seed production). In the perspective of whole plant sulphur metabolism, the requirement is the provision of adequate sulphur to optimize vegetative plant growth, and hence, reproductive potential, and ultimately to provide sulphur for seed tissues to maximize fecundity. In grain crops, the delivery of adequate sulphur to seed tissues is needed for maximal production and for quality aspects in terms of maximizing sulphur amino acid content.

At a whole plant perspective, the uptake of sulphate, its distribution and assimilation will be coordinated and balanced with the actual sulphur demand for growth. During vegetative growth, sulphur uptake is optimized for growth, and when supply is in excess, uptake mechanisms are down-regulated. Sulphate taken up in excess is loaded into vacuoles from where it can be subsequently re-mobilized. The apparent efficiency of this re-mobilization varies between

plant species and depends upon leaf maturity. In *Brassica*, the highest content of sulphate was found in the more mature leaves, and upon cessation of sulphur supply, a short lag period was apparent before this was re-mobilized (Blake-Kalff *et al*. 1998). In some cases, the rate of re-mobilization may cause young developing leaves to be sulphur deficient (Clarkson, Smith & Vandenberg 1983); however, a controlled rate of redistribution may be necessary to optimize availability in the whole plant context. In wheat and soybean, the patterns of redistribution have been extensively documented (summarized in Anderson & Fitzgerald 2003), although the roles of the specific transporters and the cues that control partitioning remain to be determined.

The mechanisms for the regulation of distribution of sulphate taken up by the root to the various sink tissues and then the subsequent redistribution are unclear. Although initial distribution occurs via the xylem, it is not simply related to transpiration, rather sulphate is directed to leaves approaching full expansion (Adiputra & Anderson 1992; Sunarpi & Anderson 1996a). Furthermore, this short-term redistribution is suggested not to be dependent upon sulphur status, but developmentally programmed. A crucial aspect would be xylem to phloem transfer, which might be achieved via Sultr1;3 or members of the group 2 sulphate transporters, which have been reported to be phloem localized (Yoshimoto *et al*. 2003). This mechanism to facilitate favouring younger leaves over older leaves for newly acquired sulphate would require a developmentally controlled process, possibly in the transfer cells (Gunning 1977), selectively removing sulphate from the transpiration stream in the xylem into these leaves.

Pulse-labelling studies with barley and soybean (Adiputra & Anderson 1992; Sunarpi & Anderson 1996a, b) indicate substantial redistribution of sulphur from mature leaves to younger leaves or generative tissues, even when plants are sulphur sufficient. Sulphur that is incorporated into protein during early leaf development is less mobile, and is predominantly re-mobilized only during senescence. Redistribution may be promoted by nitrogen stress (Sunarpi & Anderson 1997a, b) rather than sulphur stress (Adiputra & Anderson 1995; Sunarpi & Anderson 1996b), although ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) breakdown specifically in response to sulphur stress also occurs (Gilbert *et al*. 1997).

In addition to sulphate, sulphur is moved around the plant in the reduced form as glutathione (Rennenberg, Schmitz & Bergmann 1979) or as *S*-methylmethionine (Bourgis *et al*. 1999). Recently, a gene for a glutathione transporter has been identified, which is part of a larger family of oligopeptide transporters (Zhang *et al*. 2004) and is responsible for the cellular uptake of glutathione. Nutritional conditions will determine the relative importance of these reduced pools as a means of transporting sulphur within the plant. If sulphur supply has been low and vacuolar sulphate pools are not significant, then the relative contribution of reduced sulphur compounds, translocated in the phloem, becomes much greater. Generally, sulphate is a much larger pool than glutathione and *S*-methylmethionine, and although they may be important in sulphur delivery to sink tissues such as seed, these tissues have been shown to be fully competent for sulphate assimilation (Tabe & Droux 2001).

## SULPHATE TRANSPORTER GENE FAMILY

Transport of sulphate into the cell is considered to be a major regulated step (Vauclare *et al*. 2002). In fact, there are several distinct transport steps that are independently, but probably coordinately regulated and which serve to maintain constant cytoplasmic sulphate levels and prevent excess accumulation. To facilitate the complex movements of sulphate around the plant, the sulphate transporters themselves are encoded by a gene family consisting of 14 members in *Arabidopsis*, probably with little redundancy (Hawkesford 2003). There is clear evidence that transcription of the genes encoding the transporters involved in initial uptake at the soil–root interface, cell to cell transfer and vascular transportation as well as the vacuolar efflux transporter is controlled by plant sulphur status (Buchner *et al*. 2004a). The coordinated expression of this gene family facilitates optimum management of plant sulphate under varying conditions of supply and demand.

The sulphate transporter family comprises 14 genes in *Arabidopsis* and probably a similar number in other species (Hawkesford 2003). Based on sequence comparisons, these genes may be aligned into at least five clusters (referred to as groups 1–5, see Fig. 2). It has been proposed that the different groups represent functional sub-types; however, this may be an oversimplification. Broadly, group 1 represents high-affinity transporters, predominantly but not exclusively expressed in the roots (Smith *et al*. 1995; 1997). Many of this group are transcriptionally regulated in response to sulphur availability. A unique specific localization of one isoform in this group, AtSultr1;3, to the sieve elements–companion cell in the phloem is indicative of a specialized role in the redistribution of sulphur from source to sink tissues (Yoshimoto *et al*. 2003).

Group 2, when expressed in yeast, has a lower affinity for sulphate. Expression studies indicate a vascular tissue location, and therefore, of potential significance in considering tissue distribution of sulphate. Details of the expression patterns of the two genes found in this group differ substantially between the two plant species investigated in detail. In *Brassica*, only isoform AtSultr2;1 is expressed substantially in root, stem and leaves, while AtSultr2;2 is also expressed in roots. In *Arabidopsis*, both isoforms are expressed in roots and leaves. In *Brassica*, (Buchner *et al*. 2004a; Buchner, Takahashi & Hawkesford 2004b), the expression of AtSultr2;1 only occurs under sulphur starvation in the roots; however, in the leaves expression occurs also under sulphur-replete conditions, but is increased upon sulphur starvation. AtSultr2;2 expression in the root is increased by sulphur starvation. In *Arabidopsis*, (Takahashi *et al*. 2000), AtSultr2;1 is noticeably induced by sulphur starvation in the roots, as found in *Brassica*. There is little influence of sulphur nutrition on AtSultr2;2 expression.

 

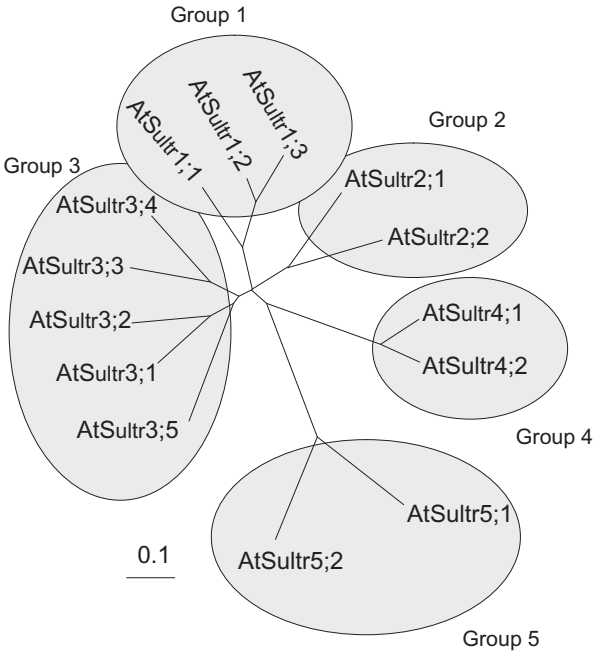


**Figure 2.** A phylogenetic tree of the *Arabidopsis* sulphate transporter gene family. Alignments of predicted protein sequences were performed using CLUSTAL W programme (Thompson *et al*. 1997) version 1.7, and the bootstrapped tree was drawn using the Treeview32 programme (Page 1996). Accession numbers: AtSultr1;1, AB018695; AtSultr1;2, AB042322; AtSultr1;3, AB049624; AtSultr2;1, AB003591; AtSultr2;2, D85416; AtSultr3;1, D89631; AtSultr3;2, AB004060; AtSultr3;3, AB023423; AtSultr3;4, B054645; AtSultr3;5, AB061739; AtSultr4;1, AB008782; AtSultr4;2, AB052775; AtSultr5;1, NP_178147; AtSultr5;2, NP_180139.

Reporter gene expression studies in *Arabidopsis* indicate phloem expression in the leaves, but xylem parenchyma expression of AtSultr2;1. By contrast, AtSultr2;2 was expressed in phloem in the roots but in the vascular bundle sheath cells and not the phloem itself of the leaves (Takahashi *et al*. 2000).

The group 3 transporters are rather enigmatic. This is a rather larger group with five examples in *Arabidopsis*, and it may be that the group should be further subdivided. An intriguing suggestion is that one isoform, AtSultr3;5, functions as a heterodimer with AtSultr2;1 (Kataoka *et al*. 2004a). The data to support this idea arise from the observation that expression of AtSultr3;5 in yeast by itself fails to catalyse sulphate transport, but contributes to uptake when co-expressed with AtSultr2;1. In addition, AtSultr3;5 is constitutively expressed in the same cells as AtSultr2;1, which is only expressed under sulphur-limiting conditions; the higher activity of the heterodimer being part of the adaptive response. A homologue of AtSultr3;5 has been described for *Lotus japonicus*, which is localized on the symbiosome membrane in the $N_2$-fixing nodule (Krusell *et al*. 2005). This transporter is essential for sulphur delivery to the bacteroids and for an efficient $N_2$ fixation. By contrast to the *Arabidopsis* isoform, the *Lotus* Sultr3;5 is able to function when expressed alone in yeast.

By contrast to the plasma membrane location of the sulphate transporters of groups 1–3, groups 4 and 5 putative sulphate transporters have been localized to the tonoplast membrane (Kataoka *et al*. 2004b) (and Buchner & Hawkesford, unpublished results). The group 4 transporters have been implicated in efflux of sulphate from the vacuole and are up-regulated by sulphur stress, thus favouring the unloading of sulphate from the vacuole. The role of the group 5 transporters has yet to be established.

A greater resolution of expression patterns of individual isoforms will be informative, particularly in the light of the complex patterns of sulphate redistribution, which are both developmentally programmed and also influenced by nutrient availability.

## REGULATION OF SULPHATE UPTAKE AND TRANSPORT AT GENE AND CELLULAR LEVELS

### Nutritional status and the regulation of sulphate influx

Many studies have taken the impact of sulphur-nutritional status on sulphate-influx capacity as a model for dissecting the regulation of sulphur nutrition. Classic physiological studies indicated an increased capacity for uptake into intact plants following a period of sulphur limitation (Lee 1982; Clarkson *et al*. 1983). Evidence that this was a function of a plasma membrane activity was shown in studies with plasma membrane vesicles isolated from control and sulphur-starved plants (Hawkesford, Davidian & Grignon 1993). It was proposed that a repression mechanism operated in which some downstream reduced sulphur compound acted to repress uptake, probably acting on the transcription of the genes for the uptake transporters (Rennenberg, Kemper & Thoene 1989). When sulphur supply becomes limiting, the levels of these compounds fall and the repression is relieved. Indirect evidence using inhibitors supported a rapid turnover of the sulphate transporter proteins and the importance of transcriptional regulation (Rennenberg *et al*. 1989; Clarkson *et al*. 1992).

An interaction with cytokinin-mediated regulation of gene expression is indicated as cytokinins down-regulate the inducible high-affinity sulphate transporters, Sultr1;1 and Sultr1;2 in *Arabidopsis* (Maruyama-Nakashita *et al*. 2004). This response is moderated in the mutant, *cytokinin response1* (*cre1*), which is deficient in a two-component cytokinin receptor. This parallels the response reported for a phosphate transporter (Franco-Zorrilla *et al*. 2002) and may act antagonistically to sugar induction of expression (Lejay *et al*. 2003; Franco-Zorrilla *et al*. 2005). Under conditions that are unfavourable for cell proliferation, transporters may be subject to a general down-regulation.

### Sulphate transporter regulation

After the cloning of the first plant sulphate transporters (Smith *et al*. 1995, 1997), a clear evidence was obtained confirming that sulphur-nutritional status had a major

impact on the transcription of the high-affinity sulphate transporters in the root. Following the removal of the sulphur supply, an increased abundance of mRNAs for high-affinity transporters was observed in parallel with decreasing tissue contents of sulphate, cysteine and glutathione (Smith *et al*. 1997). Upon the re-supply of sulphate to the medium, a re-repression occurred within hours with almost parallel decreases in mRNA abundance and transporter activity measured as influx, together with rises in internal sulphur pools. Using an antibody to an expressed C-terminal region, the levels of expression of the transporter protein in the plasma membrane have been determined and shown to parallel the observed levels of actual sulphate uptake capacity (Hawkesford & Wray 2000). Such data led to the generally accepted model that the sulphate transporter is transcriptionally regulated by the sulphur-nutritional status of the plant; however, some inconsistencies remain. In barley (Smith *et al*. 1997), the uptake capacity reached a maximum after 4 d of sulphur deprivation and even decreased after this; however, sulphate transporter mRNA abundance continued to increase. In a similar experiment with potato, the sulphate transporter mRNA abundance increased over an 8 d period; however, the measured increased uptake capacity showed only a transient rise (Hopkins *et al*. 2005).

Clearly, there is more to gene expression than measuring mRNA pools as mRNA pools, proteins and activities are all quite separate entities. This also underlines the care required when interpreting micro-array data. In addition, post-translational modifications, protein turnover and kinetic properties of the transporters will all influence transporter activity.

## Role of *O*-acetylserine (OAS)

Exogenous supply of OAS leads to an increased thiol content indicating that the supply of OAS may limit cysteine synthesis (Neuenschwander, Suter & Brunold 1991; Smith *et al*. 1997). In addition, a specific influence on the expression of gene for a sulphate transporter and for enzymes of the assimilatory pathway in a mechanism analogous with that found in bacteria is suggested. In barley, exogenous supply of the cysteine precursor, OAS, enhanced transcription, seemingly overriding the repression signal, as both cysteine and glutathione were enhanced in this treatment (Smith *et al*. 1997). In bacteria, OAS and sulphide both bind to the cysteine B (CysB) protein, a *trans*-acting factor, which modulates RNA polymerase activity; OAS enhances binding while sulphide causes a conformational change releasing binding from the promoter site. By analogy with this work on enteric bacteria (Kredich 1992), and in support of an earlier suggestion that OAS could link nitrogen and sulphur metabolism (Neuenschwander *et al*. 1991), a dual model of repression and activation was proposed (Hawkesford & Wray 2000) and is now a widely accepted model of regulation in relation to balancing sulphur metabolism to that of carbon and nitrogen. The attraction of this mechanism is that when nitrogen and carbon supply exceeds sulphur availability within the cell, OAS accumulates, and this signals to switch on processes enhancing sulphate uptake and reduction. OAS by itself is not able to fully effect control, and an additional inhibitory role of, for example, sulphide or some other reduced sulphur compounds, which can accumulate when sulphur reduction is in excess, is required. It should be noted that no CysB homologue has been identified in plants.

To further support this model, many studies have analysed OAS levels during sulphur limitation with the expectation of an elevation in OAS paralleling or anticipating an increased expression of the sulphate transporter or other sulphate-regulated genes, for example, the $\beta$-subunit of $\beta$-conglycinin (Kim *et al*. 1999). Interestingly, a thiol reductase has been implicated in regulating OAS levels (Ohkama-Ohtsu *et al*. 2004): a mutant in this gene has increased OAS levels and induced sulphur response genes, including a sulphate transporter (AtSultr2;2) and an adenosine phosphosulphate (APS) reductase (APR1). The accumulation of OAS was seen upon sulphur deprivation (Kim *et al*. 1999); however, substantially increased OAS concentrations in tissues occurred only after an extended period of sulphur deprivation and appeared to be a consequence of the deprivation, rather than part of an early response signalling pathway (Buchner *et al*. 2004a; Hopkins *et al*. 2005). A contrary explanation for the effect of exogenously applied OAS is that this overcomes endogenous controls, providing an excess of an otherwise limiting substrate that will drive flux of reduced sulphur through the reductive pathway and will lead to cysteine and glutathione accumulation as has been reported (Smith *et al*. 1997). Transcriptome profiling studies have suggested that OAS is a global regulator of large numbers of genes, specifically in many cases the same genes that are regulated by sulphur-nutritional status (Hirai *et al*. 2003). This is consistent with OAS mimicking sulphur limitation as it creates an increased demand for reduced sulphur. All of these data remain consistent with the idea that OAS accumulation reflects an imbalance of nitrogen and sulphur nutrition, rather than an early metabolic signal which the plant can use to fine-tune these pathways.

## Components involved in transcriptional control

Given the apparent importance of transcriptional regulation, a priority is the identification of components of the signal transduction pathway. Potential sulphur-responsive elements (SUREs) in promoter regions have been described, although no consensus has been shown. Soybean embryo factors (SEFs) 3 and 4 have been identified to bind to a 235 bp region of the $\beta$-conglycinin promoter, which is known to be sulphur responsive (Awazuhara *et al*. 2002). Such SEF binding sites are also present in the promoter region of serine acetyltransferase of *Citrullus vulgaris*, which has also been reported to be sulphur responsive (Saito *et al*. 1997). The *NIT3* gene (nitrilase) is highly sulphur regulated, and a region of 317 bp has been identified to contain essential sulphur-nutrition regulatory elements

 

(Kutz *et al*. 2002). A 5 bp sequence represents the core element in the SURE identified in the *AtSultr1;1* gene (Maruyama-Nakashita *et al*. 2005). This core sequence appears in the promoter regions of many sulphur-responsive genes, but not all. It also occurs in promoter regions of genes, which are not sulphur responsive, leading to the conclusion that additional, as yet unidentified, sequences are additionally required. Both the SURE and SEF elements are found upstream of the highly sulphur-responsive gene, *At5g48850* (Howarth & Hawkesford, unpublished results). The next major step is the identification of *trans*-acting factors.

### Post-translational control

In spite of the rapid responses to sulphur-nutritional status in terms of transcriptional regulation and the observed rapid protein turnover, there appear to be additional levels of regulation acting on the sulphate transporters. The carboxy-terminal region contains a sulphate transporter and anti-sigma antagonist (STAS) domain (Aravind & Koonin 2000; Shibagaki & Grossman 2004; Rouached *et al*. 2005). Mutations or deletions in this region affect function and plasma membrane targeting. The region has a phosphorylation site, and furthermore, the region may be involved in protein:protein interactions, both of which could contribute to regulation. The *in vivo* significance of these regulatory mechanisms has yet to be shown. A specific protein:protein interaction of sulphate transporters in relation to multimerization has already been referred to earlier (Kataoka *et al*. 2004a). In addition, it has been suggested that an interaction with ATP sulphurylase may play a role in regulation (Logan *et al*. 1996; Hatzfeld *et al*. 1998).

## REGULATION OF SULPHATE UPTAKE AND TRANSPORT AT WHOLE PLANT LEVEL

At whole plant level, the uptake of sulphate by the root and its distribution in the plant are driven presumably by the sulphur demand for growth at any specific developmental stage (De Kok *et al*. 2002a, b; Anderson & Fitzgerald 2003). The extrapolation of the knowledge on regulation at gene/cellular level, and the signal transduction pathways involved to that at whole plant level are rather complicated. For instance, it is still unclear whether bulk changes in gene expression of the different sulphate transporters in the root mimic an altered regulation of uptake and distribution of sulphate, or to what extent there is an interference of local cellular changes in the sulphur status, perhaps acting only in certain cells. At some point, at or prior to the endodermis, in the epidermis or cortex, transport into the symplast occurs, thus at least, the endodermis is the primary selective cell layer. Sulphate is then transferred into the xylem vessels and transported to the shoot by the transpiration stream. Sulphate transporters are presumably present in the plasma membrane of most cells, and their bulk expression responds to variation in sulphate supply (Hawkesford 2003; Buchner *et al*. 2004a, b); however, changes in expression in selected cells may have a greater influence on net flux. For example, upon sulphate deprivation, there is generally a mass induction of expression of various members of the sulphate transporters in the root, whereas the increase in the overall capacity of sulphate uptake by the root is often limited (ranging from 1.5- to 15-fold in different species) (Hawkesford 2003; Hawkesford *et al*. 2003; Buchner *et al*. 2004b).

Variation of sulphate supply generally induces multiple responses facilitating an increased sulphate uptake efficiency on a whole plant basis. In addition to the general fast induction of expression of the sulphate transporters and sulphate uptake capacity by the roots, more prolonged sulphate deprivation generally results in changes of shoot/root biomass partitioning in favour of root production (Stuiver, De Kok & Westerman 1997; Yang, Stulen & De Kok 2003, 2005; Buchner *et al*. 2004a) and root morphology by increasing the total absorptive surface of the root system (Kutz *et al*. 2002; López-Bucio, Cruz-Ramirez & Herrera-Estrella 2003). The patterns of changes in morphology upon nutrient deprivation will be mediated by nutrient-specific signal transduction pathways that sense the external and/or internal nutrient concentrations to modify root development (López-Bucio *et al*. 2003); however, there are species and varietal differences. Studies on the interaction between atmospheric and pedospheric sulphur nutrition of *Brassica* showed poor shoot to root signalling in regulation of the sulphate uptake efficiency. Plants were able to utilize foliar absorbed and metabolized $SO_2$ or $H_2S$ as the sole sulphur source for growth upon sulphate deprivation of the root. Peculiarly, the strongly induced expression of the sulphate transporters in the sulphate uptake capacity of roots (Buchner *et al*. 2004a) and the decrease in shoot:root biomass ratio of *Brassica* upon sulphate deprivation were hardly affected by $SO_2$ or $H_2S$ exposure (Yang *et al*. 2003, 2005; Buchner *et al*. 2004a). There was apparently no strict and direct shoot to root signalling for the regulation of sulphate uptake by the root and transport to the shoot in relation to the need for growth. It is unclear to what extent the external or internal sulphate concentration in the root itself is the sensing factor in the modulation of the sulphate efficiency in general. Recent data suggest that when *Brassica* was grown at a maintained 5 $\mu$M sulphate concentration (the sulphate concentration in Hoagland nutrient solution is 2000 $\mu$M), plant growth was quite normal although the sulphate content of the shoots was somewhat lower compared to plants grown at 100 or 500 $\mu$M (Posthumus, Koralewska, Stuiver & De Kok, unpublished results). In spite of this, the overall sulphate concentration in the root exceeded 5000 $\mu$M, and the sulphate uptake capacity of the roots was strongly enhanced. This means that if sulphate itself is the sensed factor, then there would need to be a huge local variation in inter- and/or intracellular distribution of sulphate in root cells.

It is often stated that after an uptake by the root, sulphate is transported to the shoot where it is reduced in the chloroplast prior to its assimilation into other organic sulphur compounds. However, Pate (1965) demonstrated that roots

were able to reduce sulphur, some of which was transported as methionine and to a lesser extent as cysteine and glutathione to the shoot. All enzymes of sulphate assimilation are present in the roots (Heiss *et al*. 1999; Lappartient *et al*. 1999; Lee & Leustek 1999; Yonekura-Sakakibara *et al*. 2000), their activity and expression (e.g. that of ATP sulphurylase and APS reductase responds to the sulphate supply) (Lappartient *et al*. 1999) and sulphate reduction most likely occur in the plastids. The production of phytochelatins in response to heavy metal stress increased sulphate assimilation and resulted in an induction of not only the expression of sulphate transporters, but also of the enzymes involved in sulphate assimilation (Heiss *et al*. 1999; Lee & Leustek 1999). However, by contrast to the reduction of nitrate (Rufty *et al*. 1986), there is still little information on the abundance and compartmentation of sulphate reduction, in particular groups of cell collectively forming the root symplast. As with nitrate reduction (Scheurwater *et al*. 2002), the proportion of sulphate reduction in the root, at least for seedlings of most herbaceous and crop plants, compared to whole plant sulphur assimilation will be restricted, because the shoot:root ratio generally exceeds a factor of 2–5. However, the abundant sulphate reduction and assimilation found in the different root cell types may be important in local tissue and whole plant modulation of sulphate uptake and transport by local signalling by, for example, the *in situ* sulphate and/or OAS concentration.

## CONTROL OF ASSIMILATORY PATHWAY FLUX AT GENE AND CELLULAR LEVELS

After many years of controversy, a plant-specific pathway of sulphate reduction and assimilation into cysteine and methionine has been defined (Fig. 3). Additional complexity arises as multi-gene families exist for most enzymes, and in many cases, specific gene products of these families are localized in individual sub-cellular compartments (Hawkesford 2005). Sulphite reductase is the exception, represented by just a single gene, and localized exclusively in the chloroplast, hence ensuring that the primary site for sulphate reduction and assimilation is in the chloroplast, where adequate ATP and reductant are available. Functions of other isoforms are not fully resolved, but are likely to be involved in providing intermediates for other pathways (Rotte & Leustek 2000), in scavenging 'leaked' metabolites, for recycling of metabolite or with specific biosynthetic roles as is the case for cysteine synthase in the mitochondrion (Warrilow & Hawkesford 1998, 2000, 2002).

A major uncertainty in the pathway was resolved with the cloning and description of APS reductase (Gutierrez-Marcos *et al*. 1996; Setya, Murillo & Leustek 1996). Previously, it had been unclear as to whether there was a plant-specific APS sulphotransferase with bound sulphite intermediates or whether the pathway was analogous to the bacterial pathway and included phosphoadenosine phosphosulphate (PAPS) as an intermediate (Kopriva & Koprivova 2004). It is now clear that APS reductase is the APS sulphotransferase, and reduces APS to produce free

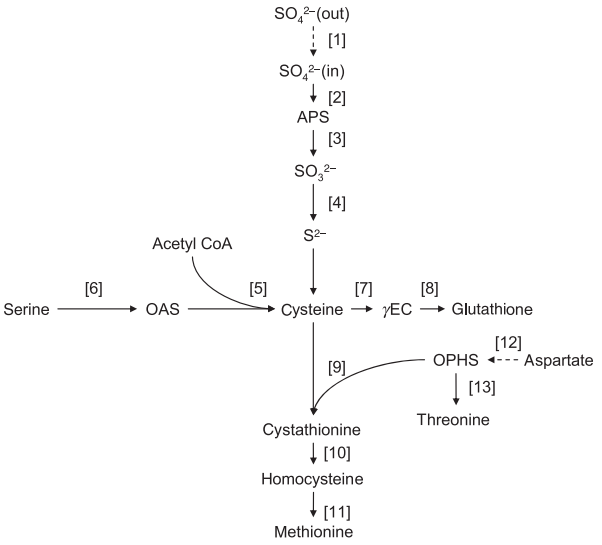


**Figure 3.** The pathways of cysteine, glutathione and methionine biosyntheses. Key to enzymes involved: [1] sulphate transporters; [2] ATP sulphurylase [enzyme class (EC) 2.7.7.4]; [3] adenosine phosphosulphate (APS) reductase (EC 1.8.4.9); [4] sulphite reductase (EC 1.8.7.1); [5] *O*-acetylserine (OAS) (thiol)lyase (EC 2.5.1.47); [6] serine acetyltransferase (EC 2.3.1.30); [7] γ-glutamylcysteine synthetase (EC 6.3.2.2); [8] glutathione synthetase (EC 6.3.2.3); [9] cystathionine γ-synthase (EC 4.2.99.2); [10] cystathionine β-lyase (EC 4.4.1.8); [11] methionine synthase (EC 2.1.1.14); [12] aspartate kinase (EC 2.7.2.4), aspartate semialdehyde dehydrogenase (EC 1.2.1.11), homoserine dehydrogenase (EC 1.1.1.3), homoserine kinase (EC 2.7.1.39); [13] threonine synthase (EC 4.2.99.2). CoA, coenzyme A; γEC, γ-glutamylcysteine; OPHS, *O*-phosphohomoserine.

sulphite directly. PAPS is part of a biosynthetic pathway branch that supplies sulphur for sulphotransferase catalysed reactions (Klein & Papenbrock 2004). APS reductase has been shown to have a major contribution to the control of pathway flux (Vauclare *et al*. 2002), that over-expression increases flux (Tsakraklides *et al*. 2002) and the expression of the respective gene is controlled by sulphate status (Hopkins *et al*. 2004).

### Cysteine synthase complex as a molecular sensor of sulphur status, controlling flux to cysteine

Cysteine synthesis is catalysed by cysteine synthase, a dissociable complex of OAS (thiol)lyase (OASTL) and serine acetyl transferase (SAT). The two component enzymes form a reversible complex comprising a homotetramer of SAT and two dimers of OASTL. The respective activities of the two components are dependent upon their association state, which in turn is controlled by the free concentration of OAS. In effect, flux through this part of the pathway is effectively controlled by OAS concentration, which varies depending upon the sulphur supply. Hence, the dissociable complex becomes a sensor (Fig. 4). At high levels of OAS (which occur under sulphur-limiting conditions), the complex dissociates and SAT are inactivated, thus prevent-

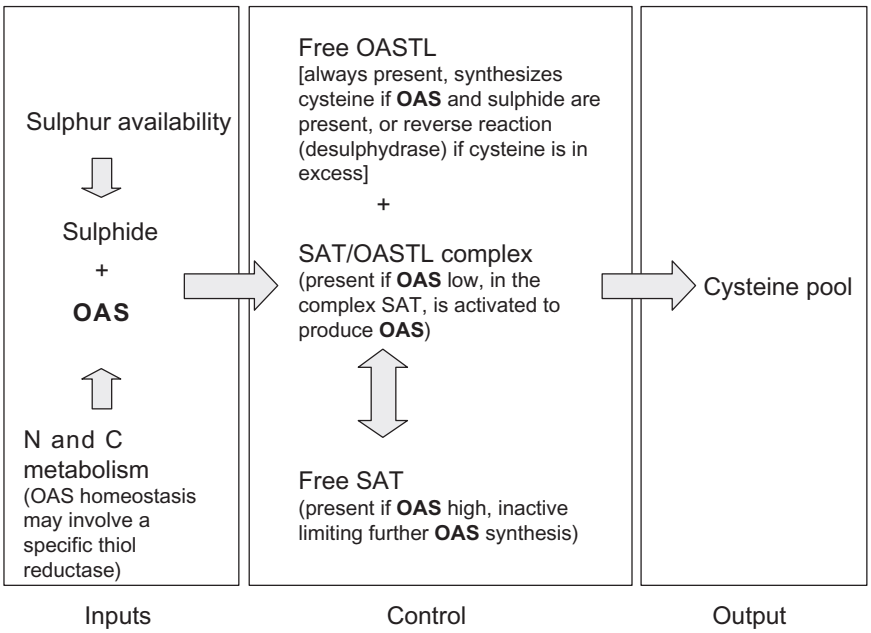


**Figure 4.** Regulation of flux by the *O*-acetylserine (OAS) (thiol)lyase (OASTL)/serine acetyl transferase (SAT) complex. Cysteine pools are regulated by the activity of the SAT/OASTL complex (Berkowitz *et al*. 2002; Droux *et al*. 1998; Wirtz *et al*. 2004). OAS concentrations will depend on relative fluxes through the sulphate reduction pathway, and nitrogen and carbon metabolism. A thiol reductase may be involved in regulating this pool size (Ohkama-Ohtsu *et al*. 2004). High levels of OAS will cause disruption of the complex, and SAT becomes inactivated, preventing further OAS accumulation. OASTL, which is present in excess, synthesizes cysteine in the free form when sulphide and OAS are available. It has been proposed that if cysteine is overproduced, for example, because of weak sink strength, then the OASTL may act as a desulphydrase (Riemenschneider *et al*. 2005a).

ing further OAS synthesis. By contrast, OASTL has maximal activity in the un-dissociated state, and with the abundant OAS supply will efficiently 'mop up' any sulphide and maximize cysteine synthesis under the sulphur-limiting conditions. When the free OAS concentration is depleted, for example, with an abundant sulphur supply, the complex re-associates activate SAT and favour OAS production. Uniquely, and by contrast to bacterial SAT, many plant SATs are cysteine feedback insensitive (Noji *et al*. 1998; Urano *et al*. 2000). However, for the cysteine-sensitive isoforms, negative feedback of cysteine on SAT prevents runaway synthesis of OAS when sulphide is plentiful, and flux to cysteine would be high, conditions under which no OAS accumulation would occur to initiate the control at the level of SAT inhibition by the association/dissociation mechanism.

An additional element to this regulation of metabolite pools has been proposed with respect to the regulation of cysteine pools. In transgenic potato lines in which OASTL was down-regulated, $H_2S$-evolving capacity was decreased whilst unexpectedly the thiol levels were increased. The conclusion was that the free OASTL has a role not only in *de novo* cysteine synthesis, but also in homeostasis acting as a desulphydrase (Riemenschneider *et al*. 2005a).

### Methionine

Methionine is an important sink for sulphur, required both as a constituent of proteins and as a methyl group donor or precursor for metabolites such as ethylene, polyamines and dimethylsulphoniopropionate. Methionine biosynthesis represents a convergence point of the sulphur-assimilatory pathway and the aspartate-derived amino acid family biosynthetic pathways. The control of flux of sulphur to methionine, and hence, the control of methionine pools appears to differ between plant species (Hesse & Hoefgen 2003). In potato and *Arabidopsis*, *S*-adenosylmethionine (SAM) feedback activates the threonine synthase enzyme in a direct allosteric fashion (Curien *et al*. 1998), enhancing flux of the common substrate, *O*-phosphohomoserine (OPHS), to the threonine branch and restricting flow for methionine biosynthesis. In *Arabidospis* but not in potato, there is further negative regulation, in a post-transcriptional mechanism, of expression of cystathionine γ-synthase by an end product of the pathway, probably either methionine or SAM (Inaba *et al*. 1994; Chiba *et al*. 1999; Kim & Leustek 2000; Hacham, Avraham & Amir 2002; Hesse & Hoefgen 2003). Both methionine and threonine syntheses may be limited by homoserine availability (Lee *et al*. 2005), the precursor for OPHS. This would take a level of control to the lysine branch point in the aspartate pathways.

## CONTROL OF ASSIMILATORY PATHWAY FLUX AT WHOLE PLANT LEVEL

As with sulphate uptake and distribution, the assimilation of sulphate in plants is driven and balanced with a demand for growth and development. The overall sulphur flux in the plant at any stage of development can be estimated by multiplying biomass production with time by the plant sulphur content (De Kok *et al*. 2002a, b). The actual flux of the sulphur assimilatory pathway may be estimated if the organic sulphur content is used in the calculation. There is a large variation in sulphur demand and assimilatory pathway flux between plant species, which in addition, is strongly affected by environmental (growth) conditions. At optimal growth conditions, the sulphur assimilatory pathway flux of different crop plants ranges from 0.03 to 0.2 $\mu$mol $g^{-1}$ plant fresh weight (FW) $h^{-1}$ (De Kok *et al*. 2002b; Durenkamp & De Kok 2004). It is evident that

plants have, to some extent, a physiological plasticity to modulate the sulphur flux in order to adapt to an altered sulphur demand and limited or excess sulphur supply by changing the level and expression of sulphate transporters and the enzymes of the sulphur assimilatory pathway.

Despite the tremendous progress in the control of assimilatory pathway flux at gene/cellular level, an insight on the regulation of the sulphur assimilatory pathway in relation to the regulation of the uptake and distribution of sulphur at whole plant level (e.g. shoot:root and source:sink signalling) is largely unclear. Moreover, conclusions of changes in fluxes and regulatory control are often based on observed variation in levels of free specific sulphur metabolites, ignoring the predominant proportion found in proteins, which as cysteine and methionine represent, up to 70% of the total sulphur content of plants. Many questions raised more than a decade ago (Stulen & De Kok 1993) are still valid for the interpretation of sulphur assimilatory pathway regulation at whole plant level: (1) how are the overall reduction and assimilation of sulphate controlled so that under non-limiting conditions of sulphur supply and growth conditions, sulphate reduction reaches a maximum and no more reduced sulphur is accumulated; (2) how does the cell (both root and shoot) control the fluxes of sulphate into the chloroplast (plastid) and vacuole; and (3) how does the sulphur demand for growth regulate the uptake and transport of sulphate, and what signal transduction pathway is involved?

The level of metabolites, which play an important role in the regulation of the sulphate assimilatory pathway, is quite dynamic and has been shown to be dependent on the environmental conditions; however, their sub-cellular distribution is largely unclear. For example, if plants are exposed to atmospheric sulphur gases such as $H_2S$, these gases are assimilated and utilized as a sulphur source. However, exposure also generally results in an increased size and change in composition of the thiol pool of the shoot, dependent on the $H_2S$ concentration, leaf age, the exposure temperature, the level of sulphur nutrition and which varies strongly between species. In some species, exposure resulted in a slight increase in the thiol content of the roots. Upon $H_2S$ exposure, the glutathione level in the shoot may reach a new steady-state level up to threefold greater than that of unexposed plants, and the levels of cysteine may increase more than 30-fold, without negative effects on plant growth (De Kok *et al*. 2002a; Durenkamp & De Kok 2004; Riemenschneider *et al*. 2005b). Apparently, the thiol accumulation reflects a slight overload of the reduced sulphur supply, but its steady-state level is beyond strict regulatory control. The levels of the precursor of cysteine, OAS and also that of *N*-acetylserine in the shoots were strongly decreased upon prolonged $H_2S$ exposure (Buchner *et al*. 2004a; Riemenschneider *et al*. 2005b).

### Stress-induced demands for sulphur

A number of stresses appear to increase the plant demand for reduced sulphur, notable amongst these are oxidative stress and heavy metal exposure. An increased expression of sulphate transporters and enzymes of the assimilatory pathway is seen in response to stress (Schäfer, Haag-Kerwer & Rausch 1998; Heiss *et al*. 1999; Vanacker, Carver & Foyer 2000; Harada *et al*. 2002; Howarth *et al*. 2003a). Transgenic lines with increased fluxes to cysteine, and hence, protective and/or metal-chelating compounds such as glutathione and phytochelatins may contribute to an improved tolerance of plants to these stresses (Błaszczyk, Brodzik & Sirko 1999; Domínguez-Solís *et al*. 2001, 2004; Noji *et al*. 2001; Howarth *et al*. 2003b). However, these compounds, even at high stress levels, generally account for only a minor proportion of the total plant sulphur content. It is assumed that modulation of glutathione levels (e.g. upon stress conditions) would require a higher sulphate uptake and assimilatory sulphur flux; however, glutathione accounts only for a minor proportion (about 2%) of the total organic sulphur content and assimilatory sulphur flux (De Kok & Stulen 1993; Stulen & De Kok 1993). Alternatively, an enhanced glutathione level upon stress exposure might be the consequence of an imbalance between the rate of sulphur assimilation and protein synthesis at a stress-induced hampered growth (Stuiver, De Kok & Kuiper 1992).

Several recent studies have underlined the importance of sulphur nutrition in plant defence. Many plant anti-pathogen compounds are sulphur containing, including thionins (Thomma, Cammue & Thevissen 2002), alliins (Jones *et al*. 2004) and glucosinolates (Griffiths, Birch & Hillman 1998). Profiles of glucosinolates can be modified by pathogen infection (Mikkelsen *et al*. 2003). Recent observations have shown that elemental sulphur is deposited in xylem parenchyma upon infection with *Verticillium dahliae* in both *Theobroma cacao* and in tomato, and appears to limit the spread of infection (Williams *et al*. 2002; Howarth *et al*. 2003a; Cooper & Williams 2004). The biosynthetic pathway for elemental sulphur production is not known. A general mechanism of importance for pathogen resistance may be the release of sulphide from desulphydrase reactions in response to infection (Bloem *et al*. 2004; Riemenschneider *et al*. 2005b). Maintaining these defence reactions places a high demand on the plant sulphur pathway and requires an optimum sulphur nutritional availability.

## SULPHUR METABOLISM AS PART OF A METABOLIC NETWORK

There are several studies employing transcriptome analysis to study global responses to sulphur deprivation. In each case, one or more discrete time points, in some cases together with a sulphur re-supply and/or OAS treatment, have been studied (Hirai *et al*. 2003; Nikiforova *et al*. 2003). In one case, a mutant lacking the Sultr1;2 sulphate transporter, which as a consequence had reduced uptake capacity and sulphur pools, was analysed (Maruyama-Nakashita *et al*. 2003). In this instance, a steady-state reduced sulphur availability was achieved, rather than the typical experimental non-steady state or highly stressed situation. Tran-

scriptome profiles indicated the up-regulation of antioxidant mechanisms in response to the reduced glutathione availability. Generally in transcriptome studies, large numbers of genes have been shown to vary in their expression in terms of mRNA pool sizes. The regulated genes may be placed into functional categories (Wawrzyńska *et al*. 2005), or into pathways and defined areas of metabolism (Nikiforova *et al*. 2003, 2004). In most instances, hypotheses may be made to implicate the respective processes in the adaptive response. Large numbers of regulated genes might be expected given the cascade of responses expected as a consequence of sulphur limitation (Fig. 1).

Metabolite profiling provides a similar picture of interacting pathways responding to an external stimulus such as sulphur deprivation (Nikiforova *et al*. 2005). A coordinating network of adaptation is revealed, which is suggested to result in a re-balancing of plant metabolism as a whole, so called 'systems re-balancing'. Analysis of multiple time points aids in distinguishing early and late responses in which resources are channelled to optimize metabolism. There is an initial accumulation of certain nitrogen-rich compounds, but ultimately many catabolic pathways are switched on.

An ideal approach is to perform both transcriptome and metabolome analyses, and to combine these data sets (Hirai *et al*. 2004, 2005). Analysing responses to both nitrogen and sulphur limitation reveals the extent of both parallel responses to these nutrient deficiencies (e.g. photosynthetic pathways, nitrate reductase and glucosinolate metabolism) and those specific for the individual nutrient limitation (Hirai *et al*. 2004). In such studies, often the imposed deficiencies are quite severe and will result in complex responses (Fig. 1).

A goal of the 'omic' approaches is the elucidation of these signalling components; however, these may not be differentially expressed themselves but must be inferred by association of interacting pathways or genes. For example, a gene encoding the v-myb myeloblastosis viral oncogene homologue (MYB) transcription factor, *production of anthocyanin pigment1* (*PAP1*), was identified because of co-expression with anthocyanin biosynthetic genes, which were all co-regulated by sulphur availability (Hirai *et al*. 2005; Tohge *et al*. 2005). The identification of non-regulated components is far more demanding.

## SUMMARY

Sulphur is an important nutrient for plant growth and health. Optimizing its assimilation into the many compounds involved in specific aspects of metabolism, as well as coordinating its incorporation into amino acids and proteins by balancing availability and sink demands, requires a complex network of interacting aspects of plant metabolism. Furthermore, sulphur pools are managed, mobilized and distributed through development to optimize fecundity. Understanding the underlying mechanisms at gene, cellular and whole plant levels may enable us to produce crops with improved quality and resistance to stress. As a paradigm for nutritional management, ideas concerning sulphur fertilizers and their efficient use may be helpful for optimizing fertilizer use efficiency in general.

## ACKNOWLEDGMENTS

M.J.H. is sponsored by grants from the Biotechnology and Biological Sciences Research Council (BBSRC) and Department for Environment, Food and Rural Affairs (DEFRA). Rothamsted Research receives grant-aided support from the BBSRC of the UK.

## REFERENCES

Adiputra I.G.K. & Anderson J.W. (1992) Distribution and redistribution of sulfur taken up from nutrient solution during vegetative growth in barley. *Physiologia Plantarum* **85,** 453–460.

Adiputra I.G.K. & Anderson J.W. (1995) Effect of sulphur nutrition on redistribution of sulphur in vegetative barley. *Physiologia Plantarum* **95,** 643–650.

Anderson J.W. & Fitzgerald M.A. (2003) Sulphur distribution and redistribution. In *Sulphur in Plants* (eds Y.P. Abrol & A. Ahmad), pp. 113–134. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Aravind L. & Koonin E.V. (2000) The STAS domain – a link between anion transporters and antisigma-factor antagonists. *Current Biology* **10,** R53–R55.

Awazuhara M., Kim H., Goto D.B., Matsui A., Hayashi H., Chino M., Kim S.G., Naito S. & Fujiwara T. (2002) A 235-bp region from a nutritionally regulated soybean seed-specific gene promoter can confer its sulfur and nitrogen response to a constitutive promoter in aerial tissues of *Arabidopsis thaliana*. *Plant Science* **163,** 75–82.

Berkowitz O., Wirtz M., Wolf A., Kuhlmann J. & Hell R. (2002) Use of biomolecular interaction analysis to elucidate the regulatory mechanism of the cysteine synthase complex from *Arabidopsis thaliana*. *Journal of Biological Chemistry* **277,** 30629–30634.

Bick J.A. & Leustek T. (1998) Plant sulfur metabolism – the reduction of sulfate to sulfite. *Current Opinion in Plant Biology* **1,** 240–244.

Blake-Kalff M.M.A., Harrison K.R., Hawkesford M.J., Zhao F.J. & McGrath S.P. (1998) Distribution of sulfur within oilseed rape leaves in response to sulfur deficiency during vegetative growth. *Plant Physiology* **118,** 1337–1344.

Blake-Kalff M.M.A., Hawkesford M.J., Zhao F.J. & McGrath S.P. (2000) Diagnosing sulfur deficiency in field-grown oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). *Plant and Soil* **225,** 95–107.

Błaszczyk A., Brodzik R. & Sirko A. (1999) Increased resistance to oxidative stress in transgenic tobacco plants overexpressing bacterial serine acetyltransferase. *Plant Journal* **20,** 237–243.

Bloem E., Riemenschneider A., Volker J., Papenbrock J., Schmidt A., Salac I., Haneklaus S. & Schnug E. (2004) Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. *Journal of Experimental Botany* **55,** 2305–2312.

Bourgis F., Roje S., Nuccio M.L., *et al.* (1999) *S*-methylmethionine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. *Plant Cell* **11,** 1485–1497.

Buchner P., Stuiver C.E.E., Westerman S., Wirtz M., Hell R., Hawkesford M.J. & De Kok L.J. (2004a) Regulation of sulfate uptake and expression of sulfate transporter genes in *Brassica*

*oleracea* as affected by atmospheric $H_2S$ and pedospheric sulfate nutrition. *Plant Physiology* **136,** 3396–3408.

Buchner P., Takahashi H. & Hawkesford M.J. (2004b) Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. *Journal of Experimental Botany* **55,** 1765–1773.

Chiba Y., Ishikawa M., Kijima F., Tyson R.H., Kim J., Yamamoto A., Nambara E., Leustek T., Wallsgrove R.M. & Naito S. (1999) Evidence for autoregulation of cystathionine gamma-synthase mRNA stability in *Arabidopsis*. *Science* **286,** 1371–1374.

Clarkson D.T., Smith F.W. & Vandenberg P.J. (1983) Regulation of sulfate transport in a tropical legume, *Macroptilium atropurpureum*, cv Siratro. *Journal of Experimental Botany* **34,** 1463–1483.

Clarkson D.T., Saker L.R. & Purves J.V. (1989) Depression of nitrate and ammonium transport in barley plants with diminished sulfate status – evidence of co-regulation of nitrogen and sulfate intake. *Journal of Experimental Botany* **40,** 953–963.

Clarkson D.T., Hawkesford M.J., Davidian J.-C. & Grignon C. (1992) Contrasting responses of sulfate and phosphate-transport in barley (*Hordeum vulgare* L.) roots to protein-modifying reagents and inhibition of protein synthesis. *Planta* **187,** 306–314.

Cooper R.M. & Williams J.S. (2004) Elemental sulphur as an induced antifungal substance in plant defence. *Journal of Experimental Botany* **55,** 1947–1953.

Curien G., Job D., Douce R. & Dumas R. (1998) Allosteric activation of *Arabidopsis* threonine synthase by *S*-adenosylmethionine. *Biochemistry* **37,** 13212–13221.

De Kok L.J., Castro A., Durenkamp M., Stuiver C.E.E., Westerman S., Yang L. & Stulen I. (2002a) Sulphur in plant physiology. In *Proceedings No 500* pp. 1–26, The International Fertiliser Society, York UK.

De Kok L.J., Stuiver C.E.E., Westerman S. & Stulen I. (2002b) Elevated levels of hydrogen sulfide in the plant environment: nutrient or toxin. In *Air Pollution and Biotechnology in Plants* (eds K. Omasa, H. Saji, S. Youssefian & N. Kondo), pp. 201–213. Springer-Verlag, Tokyo, Japan.

De Kok L.J. & Stulen I. (1993) Functions of glutathione in plants under oxidative stress. In *Sulfur Nutrition and Assimilation in Higher Plants; Regulatory, Agricultural and Environmental Aspects* (eds L.J. De Kok, I. Stulen, H. Rennenberg, C. Brunold & W.E. Rauser), pp. 125–138. SPB Academic Publishing, The Hague, The Netherlands.

Domínguez-Solís J.R., Gutiérrez-Alcalá G., Romero L.C. & Gotor C. (2001) The cytosolic *O-acetylserine (thiol) lyase* gene is regulated by heavy metals and can function in cadmium tolerance. *Journal of Biological Chemistry* **276,** 9297–9302.

Domínguez-Solís J.R., López-Martín M.C., Ager F.J., Ynsa M.D., Romero L.C. & Gotor C. (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant Biotechnology Journal* **2,** 469–476.

Droux M. (2004) Sulfur assimilation and the role of sulfur in plant metabolism: a survey. *Photosynthesis Research* **79,** 331–348.

Droux M., Ruffet M.L., Douce R. & Job D. (1998) Interactions between serine acetyltransferase and *O*-acetylserine (thiol) lyase in higher plants – structural and kinetic properties of the free and bound enzymes. *European Journal of Biochemistry* **255,** 235–245.

Durenkamp M. & De Kok L.J. (2004) Impact of pedospheric and atmospheric sulphur nutrition on sulphur metabolism of *Allium cepa* L., a species with a potential sink capacity for secondary sulphur compounds. *Journal of Experimental Botany* **55,** 1821–1830.

Franco-Zorrilla J.M., Martín A.C., Solano R., Rubio V., Leyva A. & Paz-Ares J. (2002) Mutations at CRE1 impair cytokinin-induced repression of phosphate starvation responses in *Arabidopsis*. *Plant Journal* **32,** 353–360.

Franco-Zorrilla J.M., Martín A.C., Leyva A. & Paz-Ares J.P. (2005) Interaction between phosphate-starvation, sugar, and cytokinin signaling in *Arabidopsis* and the roles of cytokinin receptors CRE1/AHK4 and AHK3. *Plant Physiology* **138,** 847–857.

Gilbert S.M., Clarkson D.T., Cambridge M., Lambers H. & Hawkesford M.J. (1997) $SO_4^{2-}$ deprivation has an early effect on the content of ribulose-1,5-bisphosphate carboxylase/oxygenase and photosynthesis in young leaves of wheat. *Plant Physiology* **115,** 1231–1239.

Griffiths D.W., Birch A.N.E. & Hillman J.R. (1998) Antinutritional compounds in the Brassicaceae: analysis, biosynthesis, chemistry and dietary effects. *Journal of Horticultural Science and Biotechnology* **73,** 1–18.

Gunning B.E.S. (1977) Transfer cells and their roles in transport of solutes in plants. *Science Progress* **64,** 539–568.

Gutierrez-Marcos J.F., Roberts M.A., Campbell E.I. & Wray J.L. (1996) Three members of a novel small gene-family from *Arabidopsis thaliana* able to complement functionally an *Escherichia coli* mutant defective in PAPS reductase activity encode proteins with a thioredoxin-like domain and 'APS reductase' activity. *Proceedings of the National Academy of Sciences of the USA* **93,** 13377–13382.

Hacham Y., Avraham T. & Amir R. (2002) The *N*-terminal region of *Arabidopsis* cystathionine gamma-synthase plays an important regulatory role in methionine metabolism. *Plant Physiology* **128,** 454–462.

Harada E., Yamaguchi Y., Koizumi N. & Sano H. (2002) Cadmium stress induces production of thiol compounds and transcripts for enzymes involved in sulfur assimilation pathways in *Arabidopsis*. *Journal of Plant Physiology* **159,** 445–448.

Hatzfeld Y., Cathala N., Grignon C. & Davidian J.C. (1998) Effect of ATP sulfurylase overexpression in bright yellow 2 tobacco cells – regulation of ATP sulfurylase and $SO_4^{2-}$ transport activities. *Plant Physiology* **116,** 1307–1313.

Hawkesford M.J. (2003) Transporter gene families in plants: the sulphate transporter gene family – redundancy or specialization? *Physiologia Plantarum* **117,** 155–163.

Hawkesford M.J. (2005) Sulphur. In *Plant Nutritional Genomics* (eds M.R. Broadley & P.J. White), pp. 87–111. Blackwell Publishing, Oxford, UK.

Hawkesford M.J., Davidian J.-C. & Grignon C. (1993) Sulfate proton cotransport in plasma-membrane vesicles isolated from roots of *Brassica napus* L. – increased transport in membranes isolated from sulfur-starved plants. *Planta* **190,** 297–304.

Hawkesford M.J., Buchner P., Hopkins L. & Howarth J.R. (2003) The plant sulfate transporter family: specialized functions and intergration with whole plant nutrition. In *Sulfur Transport and Assimilation in Plants: Regulation, Interaction and Signaling* (eds J.-C. Davidian, D. Grill, L.J. De Kok, I. Stulen, M.J. Hawkesford, E. Schnug & H. Rennenberg), pp. 1–10. Backhuys Publishers, Leiden, The Netherlands.

Hawkesford M.J. & Wray J.L. (2000) Molecular genetics of sulphate assimilation. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **33,** 159–223.

Heiss S., Schäfer H.J., Haag-Kerwer A. & Rausch T. (1999) Cloning sulfur assimilation genes of *Brassica juncea* L. cadmium differentially affects the expression of a putative low-affinity sulfate transporter and isoforms of ATP sulfurylase and APS reductase. *Plant Molecular Biology* **39,** 847–857.

Hell R. & Hillebrand H. (2001) Plant concepts for mineral acquisition and allocation. *Current Opinion in Biotechnology* **12,** 161–168.

Hesse H. & Hoefgen R. (2003) Molecular aspects of methionine biosynthesis. *Trends in Plant Science* **8,** 259–262.

 

Hirai M.Y., Fujiwara T., Awazuhara M., Kimura T., Noji M. & Saito K. (2003) Global expression profiling of sulfur-starved *Arabidopsis* by DNA macroarray reveals the role of *O*-acetyl-L-serine as a general regulator of gene expression in response to sulfur nutrition. *Plant Journal* **33,** 651–663.

Hirai M.Y., Yano M., Goodenowe D.B., Kanaya S., Kimura T., Awazuhara M., Arita M., Fujiwara T. & Saito K. (2004) Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* **101,** 10205–10210.

Hirai M.Y., Klein M., Fujikawa Y., *et al.* (2005) Elucidation of gene-to-gene and metabolite-to-gene networks in *Arabidopsis* by integration of metabolomics and transcriptomics. *Journal of Biological Chemistry* **280,** 25590–25595.

Hopkins L., Parmar S., Bouranis D.L., Howarth J.R. & Hawkesford M.J. (2004) Coordinated expression of sulfate uptake and components of the sulfate assimilatory pathway in maize. *Plant Biology* **6,** 408–414.

Hopkins L., Parmar S., Baszczyk A., Hesse H., Hoefgen R. & Hawkesford M.J. (2005) *O*-acetylserine and the regulation of expression of genes encoding components for sulfate uptake and assimilation in potato. *Plant Physiology* **138,** 433–440.

Howarth J.R., Fourcroy P., Davidian J.-C., Smith F.W. & Hawkesford M.J. (2003a) Cloning of two contrasting high-affinity sulfate transporters from tomato induced by low sulfate and infection by the vascular pathogen *Verticillium dahliae*. *Planta* **218,** 58–64.

Howarth J.R., Domínguez-Solís J.R., Gutierréz-Alcalá G., Wray J.L., Romero L.C. & Gotor C. (2003b) The serine acetyltransferase gene family in *Arabidopsis thaliana* and the regulation of its expression by cadmium. *Plant Molecular Biology* **51,** 589–598.

Inaba K., Fujiwara T., Hayashi H., Chino M., Komeda Y. & Naito S. (1994) Isolation of an *Arabidopsis thaliana* mutant, Mto1, that overaccumulates soluble methionine – temporal and spatial patterns of soluble methionine accumulation. *Plant Physiology* **104,** 881–887.

Jones M.G., Hughes J., Tregova A., Milne J., Tomsett A.B. & Collin H.A. (2004) Biosynthesis of the flavour precursors of onion and garlic. *Journal of Experimental Botany* **55,** 1903–1918.

Kataoka T., Hayashi N., Yamaya T. & Takahashi H. (2004a) Root-to-shoot transport of sulfate in *Arabidopsis*. Evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. *Plant Physiology* **136,** 4198–4204.

Kataoka T., Watanabe-Takahashi A., Hayashi N., Ohnishi M., Mimura T., Buchner P., Hawkesford M.J., Yamaya T. & Takahashi H. (2004b) Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in *Arabidopsis*. *Plant Cell* **16,** 2693–2704.

Kim H., Hirai M.Y., Hayashi H., Chino M., Naito S. & Fujiwara T. (1999) Role of *O*-acetyl-L-serine in the coordinated regulation of the expression of a soybean seed storage-protein gene by sulfur and nitrogen nutrition. *Planta* **209,** 282–289.

Kim J. & Leustek T. (2000) Repression of cystathionine gamma-synthase in *Arabidopsis thaliana* produces partial methionine auxotrophy and developmental abnormalities. *Plant Science* **151,** 9–18.

Klein M. & Papenbrock J. (2004) The multi-protein family of *Arabidopsis* sulphotransferases and their relatives in other plant species. *Journal of Experimental Botany* **55,** 1809–1820.

Kopriva S. & Koprivova A. (2004) Plant adenosine 5′-phosphosulphate reductase: the past, the present, and the future. *Journal of Experimental Botany* **55,** 1775–1783.

Kredich N.M. (1992) The molecular-basis for positive regulation of cys promoters in *Salmonella typhimurium* and *Escherichia coli*. *Molecular Microbiology* **6,** 2747–2753.

Krusell L., Krause K., Ott T., *et al.* (2005) The sulfate transporter SST1 is crucial for symbiotic nitrogen fixation in *Lotus japonicus* root nodules. *Plant Cell* **17,** 1625–1636.

Kutz A., Muller A., Hennig P., Kaiser W.M., Piotrowski M. & Weiler E.W. (2002) A role for nitrilase 3 in the regulation of root morphology in sulphur-starving *Arabidopsis thaliana*. *Plant Journal* **30,** 95–106.

Lappartient A.G., Vidmar J.J., Leustek T., Glass A.D.M. & Touraine B. (1999) Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound. *Plant Journal* **18,** 89–95.

Lee R.B. (1982) Selectivity and kinetics of ion uptake by barley plants following nutrient deficiency. *Annals of Botany* **50,** 429–449.

Lee S.M. & Leustek T. (1999) The affect of cadmium on sulfate assimilation enzymes in *Brassica juncea*. *Plant Science* **141,** 201–207.

Lee M., Martin M.N., Hudson A.O., Lee J., Muhitch M.J. & Leustek T. (2005) Methionine and threonine synthesis are limited by homoserine availability and not the activity of homoserine kinase in *Arabidopsis thaliana*. *Plant Journal* **41,** 685–696.

Lejay L., Gansel X., Cerezo M., Tillard P., Muller C., Krapp A., von Wiren N., Daniel-Vedele F. & Gojon A. (2003) Regulation of root ion transporters by photosynthesis: functional importance and relation with hexokinase. *Plant Cell* **15,** 2218–2232.

Leustek T. (1996) Molecular genetics of sulfate assimilation in plants. *Physiologia Plantarum* **97,** 411–419.

Leustek T., Martin M.N., Bick J.A. & Davies J.P. (2000) Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annual Review of Plant Physiology and Plant Molecular Biology* **51,** 141–165.

Leustek T. & Saito K. (1999) Sulfate transport and assimilation in plants. *Plant Physiology* **120,** 637–643.

Logan H.M., Cathala N., Grignon C. & Davidian J.C. (1996) Cloning of a cDNA encoded by a member of the *Arabidopsis thaliana* ATP sulfurylase multigene family – expression studies in yeast and in relation to plant sulfur nutrition. *Journal of Biological Chemistry* **271,** 12227–12233.

López-Bucio J., Cruz-Ramirez A. & Herrera-Estrella L. (2003) The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology* **6,** 280–287.

Maruyama-Nakashita A., Inoue E., Watanabe-Takahashi A., Yarnaya T. & Takahashi H. (2003) Transcriptome profiling of sulfur-responsive genes in *Arabidopsis* reveals global effects of sulfur nutrition on multiple metabolic pathways. *Plant Physiology* **132,** 597–605.

Maruyama-Nakashita A., Nakamura Y., Yamaya T. & Takahashi H. (2004) A novel regulatory pathway of sulfate uptake in *Arabidopsis* roots: implication of CRE1/WOL/AHK4-mediated cytokinin-dependent regulation. *Plant Journal* **38,** 779–789.

Maruyama-Nakashita A., Nakamura Y., Watanabe-Takahashi A., Inoue E., Yamaya T. & Takahashi H. (2005) Identification of a novel *cis*-acting element conferring sulfur deficiency response in *Arabidopsis* roots. *Plant Journal* **42,** 305–314.

Mikkelsen M.D., Petersen B.L., Glawischnig E., Jensen A.B., Andreasson E. & Halkier B.A. (2003) Modulation of *CYP79* genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. *Plant Physiology* **131,** 298–308.

Neuenschwander U., Suter M. & Brunold C. (1991) Regulation of sulfate assimilation by light and *O*-acetyl-L-serine in *Lemna minor* L. *Plant Physiology* **97,** 253–258.

Nikiforova V., Freitag J., Kempa S., Adamik M., Hesse H. &

Hoefgen R. (2003) Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. *Plant Journal* **33,** 633–650.

Nikiforova V.J., Gakière B., Kempa S., Adamik M., Willmitzer L., Hesse H. & Hoefgen R. (2004) Towards dissecting nutrient metabolism in plants: a systems biology case study on sulphur metabolism. *Journal of Experimental Botany* **55,** 1861–1870.

Nikiforova V.J., Kopka J., Tolstikov V., Fiehn O., Hopkins L., Hawkesford M.J., Hesse H. & Hoefgen R. (2005) Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of *Arabidopsis* plants. *Plant Physiology* **138,** 304–318.

Noji M., Inoue K., Kimura N., Gouda A. & Saito K. (1998) Isoform-dependent differences in feedback regulation and subcellular localization of serine acetyltransferase involved in cysteine biosynthesis from *Arabidopsis thaliana*. *Journal of Biological Chemistry* **273,** 32739–32745.

Noji M., Saito M., Nakamura M., Aono M., Saji H. & Saito K. (2001) Cysteine synthase overexpression in tobacco confers tolerance to sulfur-containing environmental pollutants. *Plant Physiology* **126,** 973–980.

Ohkama-Ohtsu N., Kasajima I., Fujiwara T. & Naito S. (2004) Isolation and characterization of an *Arabidopsis* mutant that overaccumulates *O*-acetyl-L-serine. *Plant Physiology* **136,** 3209–3222.

Page R.D.M. (1996) Tree view: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12,** 357–358.

Pate J.S. (1965) Roots as organs of assimilation of sulfate. *Science* **149,** 547–548.

Rennenberg H., Schmitz K. & Bergmann L. (1979) Long-distance transport of sulfur in *Nicotiana tabacum*. *Planta* **147,** 57–62.

Rennenberg H., Kemper O. & Thoene B. (1989) Recovery of sulfate transport into heterotrophic tobacco cells from inhibition by reduced glutathione. *Physiologia Plantarum* **76,** 271–276.

Riemenschneider A., Riedel K., Hoefgen R., Papenbrock J. & Hesse H. (2005a) Impact of reduced *O*-acetylserine (thiol) lyase isoform contents on potato plant metabolism. *Plant Physiology* **137,** 892–900.

Riemenschneider A., Nikiforova V., Hoefgen R., De Kok L.J. & Papenbrock J. (2005b) Impact of elevated $H_2S$ on metabolite levels, activity of enzymes and expression of genes involved in cysteine metabolism. *Plant Physiology and Biochemistry* **43,** 473–483.

Rotte C. & Leustek T. (2000) Differential subcellular localization and expression of ATP sulfurylase and 5′-adenylylsulfate reductase during ontogenesis of *Arabidopsis* leaves indicates that cytosolic and plastid forms of ATP sulfurylase may have specialized functions. *Plant Physiology* **124,** 715–724.

Rouached H., Berthomieu P., El Kassis E., Cathala N., Catherinot V., Labesse G., Davidian J.-C. & Fourcroy P. (2005) Structural and functional analysis of the C-terminal STAS (sulfate transporter and anti-sigma antagonist) domain of the *Arabidopsis thaliana* sulfate transporter SULTR1.2. *Journal of Biological Chemistry* **280,** 15976–15983.

Rufty T.W., Thomas J.F., Remmler J.L., Campbell W.H. & Volk R.J. (1986) Intercellular localization of nitrate reductase in roots. *Plant Physiology* **82,** 675–680.

Saito K. (2000) Regulation of sulfate transport and synthesis of sulfur-containing amino acids. *Current Opinion in Plant Biology* **3,** 188–195.

Saito K. (2004) Sulfur assimilatory metabolism. The long and smelling road. *Plant Physiology* **136,** 2443–2450.

Saito K., Inoue K., Fukushima R. & Noji M. (1997) Genomic structure and expression analyses of *serine acetyltransferase* gene in *Citrullus vulgaris* (watermelon). *Gene* **189,** 57–63.

Schäfer H.J., Haag-Kerwer A. & Rausch T. (1998) cDNA cloning and expression analysis of genes encoding GSH synthesis in roots of the heavy-metal accumulator *Brassica juncea* L.: evidence for Cd-induction of a putative mitochondrial gamma-glutamylcysteine synthetase isoform. *Plant Molecular Biology* **37,** 87–97.

Scheurwater I., Koren M., Lambers H. & Atkin O.K. (2002) The contribution of roots and shoots to whole plant nitrate reduction in fast- and slow-growing grass species. *Journal of Experimental Botany* **53,** 1635–1642.

Setya A., Murillo M. & Leustek T. (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5′-adenylylsulfate reductase. *Proceedings of the National Academy of Sciences of the USA* **93,** 13383–13388.

Shibagaki N. & Grossman A.R. (2004) Probing the function of STAS domains of the *Arabidopsis* sulfate transporters. *Journal of Biological Chemistry* **279,** 30791–30799.

Smith F.W., Hawkesford M.J., Prosser I.M. & Clarkson D.T. (1995) Isolation of a cDNA from *Saccharomyces cerevisiae* that encodes a high affinity sulphate transporter at the plasma membrane. *Molecular General Genetics* **247,** 709–715.

Smith F.W., Hawkesford M.J., Ealing P.M., Clarkson D.T., Vanden Berg P.J., Belcher A.R. & Warrilow A.G.S. (1997) Regulation of expression of a cDNA from barley roots encoding a high affinity sulphate transporter. *Plant Journal* **12,** 875–884.

Stuiver C.E.E., De Kok L.J. & Kuiper P.J.C. (1992) Freezing tolerance and biochemical changes in wheat shoots as affected by $H_2S$ fumigation. *Plant Physiology and Biochemistry* **30,** 47–55.

Stuiver C.E.E., De Kok L.J. & Westerman S. (1997) Sulfur deficiency in *Brassica oleracea* L.: development, biochemical characterization, and sulfur/nitrogen interactions. *Russian Journal of Plant Physiology* **44,** 505–513.

Stulen I. & De Kok L.J. (1993) Whole plant regulation of sulfate uptake and metabolism – a theoretical approach and comparison with current ideas on regulation of nitrogen metabolism. In *Sulfur Nutrition and Assimilation in Higher Plants; Regulatory, Agricultural and Environmental Aspects* (eds L.J. De Kok, I. Stulen, H. Rennenberg, C. Brunold & W.E. Rauser), pp. 77–91. SPB Academic Publishing, The Hague, The Netherlands.

Sunarpi & Anderson J.W. (1996a) Distribution and redistribution of sulfur supplied as [$^{35}$S]sulfate to roots during vegetative growth of soybean. *Plant Physiology* **110,** 1151–1157.

Sunarpi & Anderson J.W. (1996b) Effect of sulfur nutrition on the redistribution of sulfur in vegetative soybean plants. *Plant Physiology* **112,** 623–631.

Sunarpi & Anderson J.W. (1997a) Effect of nitrogen nutrition on remobilization of protein sulfur in the leaves of vegetative soybean and associated changes in soluble sulfur metabolites. *Plant Physiology* **115,** 1671–1680.

Sunarpi & Anderson J.W. (1997b) Effect of nitrogen nutrition on the export of sulphur from leaves in soybean. *Plant and Soil* **188,** 177–187.

Tabe L.M. & Droux M. (2001) Sulfur assimilation in developing lupin cotyledons could contribute significantly to the accumulation of organic sulfur reserves in the seed. *Plant Physiology* **126,** 176–187.

Takahashi H., Watanabe-Takahashi A., Smith F.W., Blake-Kalff M., Hawkesford M.J. & Saito K. (2000) The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. *Plant Journal* **23,** 171–182.

Thomma B., Cammue B.P.A. & Thevissen K. (2002) Plant defensins. *Planta* **216,** 193–202.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. & Higgins D.G. (1997) The CLUSTAL_X windows interface: flexible

 

strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25,** 4876–4882.

Tohge T., Nishiyama Y., Hirai M.Y., *et al.* (2005) Functional genomics by integrated analysis of metabolome and transcriptome of *Arabidopsis* plants over-expressing an MYB transcription factor. *Plant Journal* **42,** 218–235.

Tsakraklides G., Martin M., Chalam R., Tarczynski M.C., Schmidt A. & Leustek T. (2002) Sulfate reduction is increased in transgenic *Arabidopsis thaliana* expressing 5′-adenylylsulfate reductase from *Pseudomonas aeruginosa*. *Plant Journal* **32,** 879–889.

Urano Y., Manabe T., Noji M. & Saito K. (2000) Molecular cloning and functional characterization of cDNAs encoding cysteine synthase and serine acetyltransferase that may be responsible for high cellular cysteine content in *Allium tuberosum*. *Gene* **257,** 269–277.

Vanacker H., Carver T.L.W. & Foyer C.H. (2000) Early $H_2O_2$ accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley–powdery mildew interaction. *Plant Physiology* **123,** 1289–1300.

Vauclare P., Kopriva S., Fell D., Suter M., Sticher L., von Ballmoos P., Krähenbühl U., den Camp R.O. & Brunold C. (2002) Flux control of sulphate assimilation in *Arabidopsis thaliana*: adenosine 5′-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols. *Plant Journal* **31,** 729–740.

Warrilow A.G.S. & Hawkesford M.J. (1998) Separation, subcellular location and influence of sulphur nutrition on isoforms of cysteine synthase in spinach. *Journal of Experimental Botany* **49,** 1625–1636.

Warrilow A.G.S. & Hawkesford M.J. (2000) Cysteine synthase (*O*-acetylserine (thiol) lyase) substrate specificities classify the mitochondrial isoform as a cyanoalanine synthase. *Journal of Experimental Botany* **51,** 985–993.

Warrilow A.G.S. & Hawkesford M.J. (2002) Modulation of cyanoalanine synthase and *O*-acetylserine (thiol) lyases A and B activity by beta-substituted alanyl and anion inhibitors. *Journal of Experimental Botany* **53,** 439–445.

Wawrzyńska A., Lewandowska M., Hawkesford M.J. & Sirko A. (2005) Using a suppression subtractive library-based approach to identify tobacco genes regulated in response to short-term sulphur deficit. *Journal of Experimental Botany* **56,** 1575–1590.

Williams J.S., Hall S.A., Hawkesford M.J., Beale M.H. & Cooper R.M. (2002) Elemental sulfur and thiol accumulation in tomato and defense against a fungal vascular pathogen. *Plant Physiology* **128,** 150–159.

Wirtz M., Droux M. & Hell R. (2004) *O*-acetylserine (thiol) lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana*. *Journal of Experimental Botany* **55,** 1785–1798.

Yang L., Stulen I. & De Kok L.J. (2003) Interaction between atmospheric sulfur dioxide deposition and pedospheric sulfate nutrition in Chinese cabbage. In *Sulfur Transport and Assimilation in Plants: Regulation, Interaction and Signaling* (eds J.-C. Davidian, D. Grill, L.J. De Kok, I. Stulen, M.J. Hawkesford, E. Schnug & H. Rennenberg), pp. 363–365. Backhuys Publishers, Leiden, The Netherlands.

Yang L., Stulen I. & De Kok L.J. (2005) Sulfur dioxide: relevance of toxic and nutritional effects for Chinese cabbage. *Environmental and Experimental Botany* doi: 10.1016/1.envexobot.2005.06.002

Yonekura-Sakakibara K., Onda Y., Ashikari T., Tanaka Y., Kusumi T. & Hase T. (2000) Analysis of reductant supply systems for ferredoxin-dependent sulfite reductase in photosynthetic and nonphotosynthetic organs of maize. *Plant Physiology* **122,** 887–894.

Yoshimoto N., Inoue E., Saito K., Yamaya T. & Takahashi H. (2003) Phloem-localizing sulfate transporter, Sultr1; 3, mediates re-distribution of sulfur from source to sink organs in *Arabidopsis*. *Plant Physiology* **131,** 1511–1517.

Zhang M.Y., Bourbouloux A., Cagnac O., Srikanth C.V., Rentsch D., Bachhawat A.K. & Delrot S. (2004) A novel family of transporters mediating the transport of glutathione derivatives in plants. *Plant Physiology* **134,** 482–491.

Zhao F.J., Hawkesford M.J. & McGrath S.P. (1999) Sulphur assimilation and effects on yield and quality of wheat. *Journal of Cereal Science* **30,** 1–17.

*Received 10 August 2005; received in revised form 24 October 2005; accepted for publication 1 November 2005*

 